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(4) Protein, DNA and use thereof.

(1) Disclosed are (1) a *Xenopus laevis* bone morphogenetic protein (BMP), (2) a DNA comprising a DNA segment coding for a *Xenopus laevis* BMP, (3) a transformant bearing a DNA comprising a DNA segment coding for a *Xenopus laevis* BMP and (4) a method for preparing the *Xenopus laevis* BMP which comprises culturing the described in (3), producing and accumulating the protein in a culture, and collecting the protein thus obtained. Cells transfected or transformed with the DNA allow large amounts of the *Xenopus laevis* BMP mature peptides to be produced, which causes the advantageous production of the peptides, which promote the synthesis of proteoglycan and can also be utilized for analysis of the mechanism of organism, particularly human bone-cartilage morphogenetic reaction, and as therapeutic agents for osteoporosis.

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## PROTEIN, DNA AND USE THEREOF

### BACKGROUND OF THE INVENTION

The present invention relates to a DNA segment coding for a *Xenopus laevis* bone morphogenetic protein analogous to a bone morphogenetic protein (hereinafter referred to as BMP), a precursor protein (or a precursor polypeptide) and a mature protein (or a mature polypeptide) of the *Xenopus laevis* BMP, and a method for preparing the precursor protein and the mature protein.

In this specification, the term "precursor protein" includes a protein which includes an amino acid sequence of a mature peptide *Xenopus laevis* BMP and has all or a portion of an amino acid sequence coded with a *Xenopus laevis* BMP DNA segment at the N-terminus, the C-terminus or both terminal thereof. Recently, it has been revealed that transforming growth factor-beta (TGF-beta, TGF- $\beta$ ) having a bone morphogenetic activity not only controls cell proliferation, but also has various biological activities such as control of cell differentiation. In particular, the bone morphogenesis-promoting activity of TGF- $\beta$  has been noted, and attempts have been made to use TGF for treatment of fractures and osteoporosis, making use of the cartilage-bone induction activity thereof (M. Noda et al., *J. Endocrinology* 124, 2891-2894 (1989); M. E. Joyce et al., *J. Bone Mineral Res.* 4, S-259 (1989); and S. M. Seyedin et al., *J. Biol. Chem.* 261, 5693-5695 (1986)). More recently, however, four kinds of bone morphogenetic proteins (BMPs) which are different from one another in molecular structure have been identified as a factor promoting morphogenesis of bones and cartilages. Of these four kinds, human BMP-1, human BMP-2A, human BMP-2B and human BMP-3 are novel peptides, though they are very similar in structure to TGF- $\beta$ , and there has been a report that they induce morphogenesis of bones and cartilages when subcutaneously or intramuscularly implanted in animals (J. M. Wozney et al., *Science* 242, 1528-1534 (1989)).

The above peptides having bone morphogenetic activity are isolated and purified from bones in which the peptides are considered to be localized, or from human osteosarcoma cells (U2-OS) which are thought to produce the peptides. However, such a method has problems because the procedure is complicated and the desired peptides are obtained only in small amounts.

### SUMMARY OF THE INVENTION

Important contributions will be made to future studies and medical treatment, if a similar peptide having the bone morphogenetic activity can be collected from *Xenopus laevis* and further prepared by recombinant technique. As a result, the following information was obtained, thus arriving at the present invention.

Namely, the present inventors first succeeded in cloning five kinds of DNA coding for BMP-2A and related DNAs (*Xenopus laevis* BMPs) and subsequently three kinds of complementary DNAs, eight kinds of DNAs in total, by using a complementary DNA of a rat inhibin  $\beta$ A chain equally belonging to the TGF- $\beta$  family as a probe. Further, the present inventors identified portions of the bases of the DNAs, clarified the amino acid sequences (see formulae (I), (II), (III), (IV) and (V) of Fig. 3 and formulae (VI), (VII) and (VIII) of Fig. 4) of the *Xenopus laevis* BMPs (referred to as B9, M3, C4, A4, A5, Xbr22, Xbr23 and Xbr41), and succeeded in pioneering their mass production by recombinant technique.

In accordance with the present invention, there are provided (1) a *Xenopus laevis* BMP, (2) a DNA comprising a DNA segment coding for the *Xenopus laevis* BMP, (3) a transformant bearing the DNA containing the DNA segment coding for the *Xenopus laevis* BMP and (4) a method for preparing the *Xenopus laevis* BMP which comprises culturing the transformant described in (3), producing and accumulating a protein in a culture and collecting the protein thus obtained.

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows simplified restriction enzyme maps of DNA sequences containing *Xenopus laevis* BMP precursors or mature peptide DNA segments:  
Fig. 2(1) to 2(6) show nucleotide sequences of the DNA segments of *Xenopus laevis* BMPs, B9, M3, C4, A4, A5, BMP-2A, BMP-2B and Vgr-1, respectively, and the amino acid sequences deduced therefrom;  
Fig. 3 shows amino acid sequences of the *Xenopus laevis* BMPs deduced from the nucleotide sequences of the DNA segments shown in Figs. 2(1) to 2(5), comparing them with the amino acid sequences of known proteins having a bone morphogenetic activity; and

Fig. 4 shows amino acid sequences of the *Xenopus laevis* BMPs deduced from the nucleotide sequences of the cDNA segments shown in Figs. 2(6) to 2(8).

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The mature *Xenopus laevis* BMP of C4, one of the *Xenopus laevis* BMPs, of the present invention, which has a relationship to TGF- $\beta$  and is a peptide consisting of 98 or 114 amino acid residues, has an amino acid sequence represented by Nos. 6 to 119 or Nos. 22 to 119 of formula (III) shown in Fig. 3. The molecular weight thereof is calculated at about 25,000, excepting sugar chains, when a dimer is formed. The amino acid sequence of this peptide is different from that reported by Wozney et al. in 3 or 4 amino acid residues per molecule.

Fig. 3 shows amino acid sequences of five kinds of novel *Xenopus laevis* BMPs obtained in the present invention, comparing them with the amino acid sequences of known proteins having a bone morphogenetic activity. In these amino acid sequences, the same amino acid residue as with  $\beta$ A is represented by "\*", and an amino acid residue different from that of  $\beta$ A is represented by one letter symbol based on  $\beta$ A. CONSENSUS shown in Fig. 3 indicates amino acid residues common to all the BMPs shown in Fig. 3. The illustration of CONSENSUS results in introduction of gaps "-" in the formulae in Fig. 3. Accordingly, the number representing the precursor and mature protein portions is counted excluding these lacking portions.

Fig. 4 shows amino acid sequences of three kinds of novel *Xenopus laevis* BMPs deduced from cDNAs, subsequently discovered by the present inventors.

For DNA sequences, the DNA segments coding for the *Xenopus laevis* BMPs of the present invention correspond to the nucleotide sequences of formulae (1) to (8) (corresponding to B9, M3, C4, A4, A5, Xbr22, Xbr23 and Xbr41, respectively) shown in Fig. 2 or are portions thereof. Any functional portion can be used so long as bone morphogenetic activity is not lost. Wozney et al. reports the amino acid sequences, but does not elucidate the nucleotide sequences. As used herein the term "correspond" permits conservative additions, deletions and substitutions. Preferably, the DNA segments coding for the BMPs of the present invention have the nucleotide sequences of formulae (1) to (8).

With respect to the portion relating to the mature BMPs (the amino acid sequence represented by Nos. 15 to 130 of formula (I) shown in Fig. 3, the amino acid sequence represented by Nos. 14 to 127 of formula (II) shown in Fig. 3, the amino acid sequence represented by Nos. 6 to 119 or Nos. 22 to 119 of formula (III) shown in Fig. 3, the amino acid sequence represented by Nos. 6 to 65 of formula (IV) shown in Fig. 3, the amino acid sequence represented by Nos. 282 to 398 or Nos. 298 to 398 of formula (V) shown in Fig. 3, the amino acid sequence represented by Nos. 288 to 401 or Nos. 304 to 401 of formula (VI) shown in Fig. 4, the amino acid sequence represented by Nos. 328 to 426 of formula (VII) shown in Fig. 4), the DNA sequences of the present invention differ from the DNA sequence of TGF- $\beta$ , and therefore are novel.

As the DNA sequences coding for the BMP mature peptides of the present invention, any DNA sequences may be used as long as they contain nucleotide sequences coding for the amino acid sequences of the BMP mature peptides. For example, DNA sequences corresponding to the nucleotide sequences represented by formulae (1) to (8) or portions thereof are preferably used. More preferably the DNA sequences contain the nucleotide sequences represented by formulae (1) to (8).

The nucleotide sequences represented by formulae (1) to (8) are the *Xenopus laevis* BMP DNA sequences obtained in the present invention. Examples of the nucleotides coding for the *Xenopus laevis* BMP amino acid sequences represented by formulae (I) to (VIII) include Nos. 693 to 1040 of formula (1), Nos. 134 to 475 of formula (2), Nos. 435 to 728 of formula (3), Nos. 183 to 356 of formula (4), Nos. 149 to 328 of formula (5), Nos. 249 to 1442 of formula (6), Nos. 104 to 1306 of formula (7) and Nos. 86 to 1363 of formula (8).

An expression vector having the DNA sequence containing the nucleotide sequence coding for the BMP of the present invention can be prepared, for example, by the following process:

- (a) Messenger RNA (mRNA) is isolated from BMP-producing cells.
- (b) Single stranded complementary DNA (cDNA) is synthesized from the mRNA, followed by synthesis of double stranded DNA.
- (c) The complementary DNA is introduced in a cloning vector such as a phage or a plasmid.
- (d) Host cells are transformed with the recombinant phage or plasmid thus obtained.
- (e) After cultivation of the transformant thus obtained, the plasmid or the phage containing the desired DNA is isolated from the transformant by an appropriate method such as hybridization with a DNA probe coding for a portion of the BMP or immunoassay using an anti-BMP antibody.

(f) The desired cloned DNA sequence is cut out from the recombinant DNA.  
 (g) The cloned DNA sequence or a portion thereof is ligated downstream from a promoter in the expression vector.

The mRNAs coding for the BMPs can be obtained from various BMP-producing cells such as ROS cells.  
 Methods for preparing the mRNAs from the BMP-producing cells include the guanidine thiocyanate method [J. M. Chirgwin et al., *Biochemistry* 18, 5294 (1979)].

Using the mRNA thus obtained as a template, cDNA is synthesized by use of reverse transcriptase, for example, in accordance with the method of H. Okayama et al. [Molecular and Cellular Biology 2, 161 (1979); *ibid.* 3, 280 (1983)]. The cDNA thus obtained is introduced into the plasmid.

The plasmids into which the cDNA is introduced include, for example, pBR322 [Gene 2, 95 (1977)], pBR323 [Gene 4, 121 (1978)], pUC12 [Gene 19, 259 (1982)] and pUC13 [Gene 19, 259 (1982)], each derived from *Escherichia coli*, and pUB110 derived from *Bacillus subtilis* [Biochemical and Biophysical Research Communications 112, 678 (1983)]. However, any other plasmids can be used as long as they are replicable and growable in the host cells. Examples of the phage vectors into which the cDNA may be introduced include  $\lambda$ gt10 [R. Young and R. Davis, *Proc. Natl. Acad. Sci. U.S.A.* 80, 1194 (1983)]. However, any other phage vectors can be used as long as they are growable in the host cells.

Methods for introducing the cDNA in the plasmid include, for example, the method described in T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, p.239 (1982). Methods for introducing the cDNA in the phage vector include, for example, the method of T. V. Hyman et al. [DNA Cloning, A Practical Approach 1, 49 (1985)].

The plasmid thus obtained is introduced into the appropriate host cell such as *Escherichia* and *Bacillus*.

Examples of *Escherichia* described above include *Escherichia coli* K12DH1 [Proc. Natl. Acad. Sci. U.S.A. 80, 160 (1983)], MT03 [Nucleic Acids Research 9, 309 (1981)], JA22 [Journal of Molecular Biology 120, 517 (1978)], HB101 [Journal of Molecular Biology 41, 459 (1982)] and C600 [Genetics 39, 440 (1955)].

Examples of *Bacillus* described above include *Bacillus subtilis* M1114 [Gene 24, 255 (1983)] and 207-21 [Journal of Biochemistry 95, 87 (1984)].

Methods for transforming the host cell with the plasmid include, for example, the calcium chloride method or the calcium chloride/iodine chloride method described in T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, p.249 (1982).

When the phage vector is used, for example, the phage vector can be transduced into multiplied *Escherichia coli* using the *in vitro* packaging method.

*Xenopus laevis* cDNA libraries containing *Xenopus laevis* BMP cDNA can be obtained by numerous techniques well known in the art including purchasing them from the market, though obtainable by the methods described above. For example, the cDNA library of *Xenopus laevis* is available from Clontech Laboratories, Inc., U.S.A.

Methods for cloning the *Xenopus laevis* BMP DNA from the *Xenopus laevis* DNA library include, for example, the plaque hybridization method using phage vector  $\lambda$ charon 28A and *rel* inhibin (actin)  $\beta$ A cDNA as probes [T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, (1982)].

The *Xenopus laevis* BMP DNA thus cloned is subcloned in plasmids such as pBR322, pUC12, pUC13, pUC18, pUC118 and pUC119 to obtain the *Xenopus laevis* BMP DNA, if necessary.

The nucleotide sequence of the DNA sequence thus obtained is determined, for example, by the Maxam-Gilbert method [A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* 74, 560 (1977)] or the dideoxy method [J. Messing et al., *Nucleic Acids Research* 9, 309 (1981)], and the existence of the *Xenopus laevis* BMP DNA is confirmed in comparison with the known amino acid sequence.

As described above, the DNA sequence [Xenopus laevis BMP DNA] represented by formulae (1) to (8) coding for the *Xenopus laevis* BMPs are obtained.

Fig. 1 shows the restriction enzyme fragment maps of the DNA sequences containing the DNA segments coding for the *Xenopus laevis* BMPs obtained in Example 1 described below. Fig. 2 shows the nucleotide sequences represented by formulae (1) to (8) of the DNA sequences as determined by the dideoxy method, and Figs. 3 and 4 show the amino acid sequences represented by formulae (I) to (V) and formulae (VI) to (VIII), respectively, which were ascertained from the above nucleotide sequences.

The DNA sequence coding for the *Xenopus laevis* BMP cloned as described above can be used as it is, or after digestion with a restriction enzyme if desired, depending on the intended use.

A region intended to be expressed is cut out from the cloned DNA and ligated downstream from the promoter in a vehicle (vector) suitable for expression, whereby the expression vector can be obtained.

The DNA sequence has ATG as a translation initiating codon at the 5'-terminus thereof and may have TAA, TGA or TAG as a translation terminating codon at the 3'-terminus. The translation initiating codon and translation terminating codon may be added by use of an appropriate synthetic DNA adaptor. The promoter is further ligated in the upstream thereof for the purpose of expressing the DNA sequence.

Examples of the vectors include the above plasmids derived from *E. coli* such as pBR322, pBR325, pUC12 and pUC13, the plasmids derived from *B. subtilis* such as pUB110, pTP5 and pC184, plasmids derived from yeast such as pSH19 and pSH15, bacteriophage such as  $\lambda$ phage, and animal viruses such as retroviruses and vaccinia viruses.

As the promoters used in the present invention, any promoters are appropriate as long as they are suitable for expression in the host cells selected for the gene expression.

When the host cell used for transformation is *Escherichia*, it is preferable that a *trp* promoter, a *lac* promoter, a *recA* promoter, a *XP* promoter, a *lpp* promoter, etc. are used. When the host cell is *Bacillus*, it is preferable that a *PHOS* promoter, a *PGK* promoter, a *GAP* promoter, an *ADH* promoter, etc. are used. In particular, it is preferable that the host cell is *Escherichia* and the promoter is the *trp* promoter or the *XP* promoter.

When the host cell is an animal cell, an SV-40 derived promoter, a retrovirus promoter, a metallothionein promoter, a heat shock promoter, etc. are each usable.

An enhancer, a certain DNA sequence important for promoter activity in a cell, is also effectively used for expression.

By using the vector containing the DNA sequence coding for the *Xenopus laevis* BMP mature peptide thus constructed, the transformant is prepared.

The host cell include, for example, *Escherichia*, *Bacillus*, yeast and animal cells.

Specific examples of the above *Escherichia* and *Bacillus* include strains similar to those described above.

Examples of the above yeast include *Saccharomyces cerevisiae* AH22, AH22R<sup>-</sup>, NA87, 11A and DKD-5D.  
 Examples of animal cells include monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L cell and human FL cell.

The transformation of the above *Escherichia* is carried out, for example, according to the method described in Proc. Natl. Acad. Sci. U.S.A. 69, 2110 (1972) or Gene 17, 107 (1982).

The transformation of the above *Bacillus* is conducted, for example, according to the method described in Molecular & General Genetics 168, 111 (1979).

The transformation of the yeast is carried out, for example, according to the method described in Proc. Natl. Acad. Sci. U.S.A. 75, 1929 (1978).

The transformation of the animal cells is carried out, for example, according to the method described in Virology 52, 456 (1973).

Thus, there is obtained the transformant transformed with the expression vector containing the DNA sequence coding for the *Xenopus laevis* BMP mature peptide.

When bacterial transformants are cultured, a liquid medium is particularly suitable as a medium used for culture. Carbon sources, nitrogen sources, inorganic compounds and others necessary for growth of the transformant are contained therein. Examples of the carbon sources include glucose, dextrin, soluble starch and sucrose. Examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, soybean meal and potato extract solution. The inorganic compounds include, for example, calcium chloride, sodium dihydrogenphosphate and magnesium chloride. Yeast extract, vitamins, growth promoting factors and so on may be further added thereto.

The pH of the medium is preferably about 5 to 8.  
 As the medium used for cultivation of *Escherichia*, there is preferred, for example, M9 medium containing glucose and Casamino Acids (Miller, *Journal of Experiments in Molecular Genetics* 431-433, Cold Spring Harbor Laboratory, New York, 1972), in order to make the promoter act efficiently, a drug such as 5-iodo-uracilic acid may be added thereto if necessary.

When the host cell is *Escherichia*, the cultivation is usually carried out at about 15 to 43°C for about 3 to 24 hours, with aeration or agitation if necessary.

When the host cell is *Bacillus*, the cultivation is usually carried out at about 30 to 40°C for about 8 to 24 hours, with aeration or agitation if necessary.

When yeast transformants are cultured, there is used, for example, Burkholder minimum medium [K. L. Bostian et al., *Proc. Natl. Acad. Sci. U.S.A.* 77, 4505 (1980)] as the medium. The pH of the medium is preferably adjusted to about 5 to 8. The cultivation is usually carried out at about 20 to 35°C for about 24

to 72 hours, with aeration or agitation if necessary.

When animal cell transformants are cultured, examples of the media include MEM medium containing about 5 to 20% fetal calf serum (Science 122, 501 (1952)), DMEM medium (Vitrology 8, 398 (1953)), RPMI 1640 medium (Journal of the American Medical Association 189, 518 (1957)), and 199 medium (Proceedings of the Society for the Biological Medicine 73, 1 (1950)). The pH is preferably about 6 to 8. The cultivation is usually carried out at about 30 to 40 °C for about 15 to 60 hours, with aeration or agitation if necessary.

The above *Xenopus laevis* BMP mature peptide can be isolated and purified from the culture described above, for example, by the following method.

When the *Xenopus laevis* BMP mature peptide is to be extracted from the cultured cells, the cells are collected by methods known in the art after cultivation. Then, the collected cells are suspended in an appropriate buffer solution and disrupted by ultrasonic treatment, lysozyme and/or freeze-thawing. Thereafter, a crude extracted solution of the *Xenopus laevis* BMP mature peptide is obtained by centrifugation or filtration. The buffer solution may contain a protein denaturant such as urea or guanidine hydrochloride, or a surface-active agent such as Triton X-100.

When the *Xenopus laevis* BMP precursor protein or mature peptide is secreted in the culture solution, a supernatant is separated from the cells by methods known in the art after the conclusion of cultivation, and then collected.

The separation and purification of the *Xenopus laevis* BMP precursor protein or mature peptide contained in the culture supernatant or the extracted solution thus obtained can be performed by an appropriate combination of known separating and purifying methods. The known separating and purifying methods include methods utilizing solubility such as salt precipitation and solvent precipitation, methods mainly utilizing a difference in molecular weight such as dialysis, ultrafiltration, gel filtration, and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electric charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatography, methods utilizing a difference in hydrophobicity such as reverse phase high performance liquid chromatography and methods utilizing a difference in isoelectric point such as isoelectric-focusing electrophoresis. Methods using an antibody to a fused protein expressed by using BMP complementary DNA or DNA with *E. coli*-derived DNA lacZ can also be used.

Illustrative examples of the methods for expressing the BMP in the present invention include methods in which genes are introduced into CHO cells to produce the BMP in large amounts as described in Wang et al. Proc. Natl. Acad. Sci. U.S.A. 807, 2220-2224 (1980).

The activity of the *Xenopus laevis* BMP precursor protein or mature peptide thus formed can be measured by an enzyme immunoassay using a specific antibody. If the products have a bone morphogenetic activity, this activity may also be measured as an index.

The cells, such as animal cells or *E. coli*, transfected or transformed with the DNA sequences of the present invention allow large amounts of the *Xenopus laevis* BMP mature peptides to be produced. Hence, the production of these peptides can be advantageously achieved.

It has become clear that the *Xenopus laevis* BMP mature peptides prepared here promote the synthesis of proteoglycan which is a main component of a cartilage matrix, and the peptides can also be utilized for analysis of the mechanism of organism, particularly human bone-cartilage morphogenetic reaction, and as therapeutic agents for fracture or osteoporosis.

In such instances one would administer an effective amount of the protein to a mammal. An effective amount is the amount of protein needed to promote the synthesis of proteoglycan in cartilage cells. Typically, this ranges from 0.001 to 35 µg per kg body weight. The precise amount for a particular purpose can readily be determined empirically by the person of ordinary skill in the art based upon the present disclosure.

When one uses the protein for therapeutic purpose care is taken to purify it and render it substantially free of bactericidal and pyrogenic. This can be done by standard methods.

When the BMPs are used as therapeutic agents for fracture or osteoporosis, they can be administered parenterally in the form of solutions, injections and ointments, solely or in combination with pharmacologically acceptable additional components, such as vehicles, binders, dispersants, plasticizers or diluents.

The preferable administration forms include (1) administration of the agent to cutis surface near a diseased part, (2) injection of the agent into a diseased part, (3) discission of a diseased part followed by direct administration of the agent thereto. The preferable dose in fracture therapy for adult people is 0.1 to 2000 µg more, preferably 20 to 400 µg for adult people once a day. The preferable dose in osteoporosis for adult people is 0.1 to 200 µg once a day, for about one to 30 days. The concentration of the therapeutic agent is, preferably, 0.001 to 0.2% in the form of a solution, 0.001 to 0.2% in the form of an injection, and

0.0001 to 0.2% in the form of an ointment.

There have been described above in detail the cloning of the DNA sequences coding for the *Xenopus laevis* BMPs, the preparation of the expression vectors for the *Xenopus laevis* BMP mature peptides, the production of the transformants by using the transformants and their utility.

When nucleotides, amino acids and so on are indicated by the abbreviations in this specification and drawings, the abbreviations adopted by IUPAC-IUB Commission on Biochemical Nomenclature or commonly used in the art are employed. For example, the following abbreviations are used. When the amino acids are capable of existing as optical isomer, the L-forms are represented unless otherwise specified.

DNA : Deoxyribonucleic acid  
CDNA : Complementary deoxyribonucleic acid  
A : Adenine  
T : Thymine  
G : Guanine  
C : Cytosine  
RNA : Ribonucleic acid  
mRNA : Messenger ribonucleic acid  
dATP : Deoxyadenosine triphosphate  
dTTP : Deoxythymidine triphosphate  
dGTP : Deoxyguanosine triphosphate  
dCTP : Deoxycytidine triphosphate  
ATP : Adenosine triphosphate  
EDTA : Ethylenediaminetetraacetic acid  
SDS : Sodium dodecyl sulfate  
Gly or G : Glycine  
Ala or A : Alanine  
Val or V : Valine  
Leu or L : Leucine  
Ile or I : Isoleucine  
Ser or S : Serine  
Thr or T : Threonine  
Cys or C : Cysteine  
Met or M : Methionine  
Glu or E : Glutamic acid  
Asp or D : Aspartic acid  
Lys or K : Lysine  
Arg or R : Arginine  
His or H : Histidine  
Phe or F : Phenylalanine  
Tyr or Y : Tyrosine  
Trp or W : Tryptophan  
Pro or P : Proline  
Asn or N : Asparagine  
Gln or Q : Glutamine

With respect to the *Xenopus laevis* BMP mature peptides of the present invention, a portion of the amino acid sequence may be modified, namely there may be addition, elimination or substitution with other amino acids as long as the bone morphogenetic activity is not lost.

The present invention will hereinafter be described in detail with the following Examples. It is understood of course that these Examples are not intended to limit the scope of the invention.

Transformants *E. coli* HB101/pXa3 (coding for protein M3), *E. coli* HB101/pXa4 (coding for protein A4), *E. coli* HB101/pXa5 (coding for protein A5), *E. coli* HB101/pXa6 (coding for protein B9) and *E. coli* HB101/pXa7 (coding for protein C4) each obtained in Example 1 described below were deposited with the Institute for Fermentation, Osaka, Japan (IFO) under the accession numbers IFO 14928, IFO 14929, IFO 14930, IFO 14931 and IFO 14932, respectively, on August 28, 1989. These transformants were also deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (FRI) under the accession numbers FERM BP-2578, FERM BP-2579, FERM BP-2580, FERM BP-2581 and FERM BP-2582, respectively, on September 2, 1989.

The transformants *E. coli* HB101/pXb22 (coding for *Xenopus laevis* BMP-2A), *E. coli* HB101/pXb23

(coding for *Xenopus laevis* BMP-2B) and E. coli HB101/pXbr41 (coding for protein *Xenopus laevis* Vgr-1) each obtained in Example 2 described below were deposited with the Institute for Fermentation, Osaka, Japan (IFO) under the accession numbers IFO 15080, IFO 15081 and IFO 15082, respectively, on August 10, 1990. These transformants were also deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (FRI) under the Budapest Treaty under the accession numbers FERM BP-3066, FERM BP-3065 and FERM BP-3067, respectively, on August 16, 1990.

#### Example 1

##### Preparation of *Xenopus laevis* Liver-Derived DNA Library

##### (1) Preparation of *Xenopus laevis* Chromosome DNA

The liver (1 g) of *Xenopus laevis* was powdered in liquid nitrogen, and 10 ml of buffer (1) [100 µg/ml proteinase K, 0.5% Sarkosyl, 0.5 M EDTA (pH 8.0)] was added thereto, followed by incubation at 50 °C for 2 hours. The resulting DNA sample was treated with phenol, and then dialyzed against buffer (2) [10 mM EDTA, 10 mM NaCl, 50 mM Tris-HCl (pH 8.0)] to remove phenol. RNase was added thereto to a final concentration of 100 µg/ml, and the mixture was incubated at 37 °C for 3 hours, followed by phenol treatment twice. The aqueous layer was dialyzed against buffer (3) [1 mM EDTA, 10 mM Tris-HCl (pH 8.0)]. Thus, about 1 mg of liver-derived chromosome DNA was obtained. This DNA (10 µg) was partially cleaved with restriction enzyme *Sau3A*, and the resulting product was subjected to equilibrium density gradient centrifugation using CsCl. Fractions containing DNA fragments having lengths of 10 to 20 kb were selected and introduced into fragments obtained by cleaving phage charon 28 DNA with *Bam*HI and used as a vector. This reaction called "ligation" was conducted at 15 °C for 18 hours. The charon 28 vector into which the *Xenopus laevis* chromosome DNA was thus introduced was contained in a phage head (in vitro packaging). This procedure was carried out by using a commercial packaging kit (Gigapack Gold, Stratagene). This recombinant phage was amplified by infection with E. coli LE392. Specifically, the phage was mixed with excess LE392 to allow LE392 to adsorb the phage at 37 °C for 10 minutes. Then, the mixture was plated on NZYM medium (containing 13% agar), followed by incubation overnight.

##### (2) Screening

The total number of the phage clones was estimated to be about 1,000,000 from the number of the plaques produced in a dish. As a probe (DNA used for detection of a desired gene by hybridization), there was used rat *activin βA* cDNA [Molecular Endocrinology 1, 386-396 (1987)] labeled with 32P by a random priming method. The plaques transcribed from the dish to a nitrocellulose membrane were returned to neutrality (0.2 M Tris, 0.6 M NaCl, pH 7.4) through alkali treatment (immersion in 0.1 N NaOH, 0.6 M NaCl for 30 seconds). After completion of the treatment described above, the membrane was heated in a vacuum thermostat at 80 °C for 1 hour. After heating, the membrane was immersed in a hybridization solution (50% formamide, 5 X Denhardt's solution, 5 X SSPE, 0.1% SDS, 100 µg/ml denatured salmon sperm DNA) to incubate it at 42 °C for 4 hours. Then, the membrane was allowed to stand in the mixture solution of the above hybridization solution and the DNA probe at 60 °C overnight. This procedure was carried out in a plastic bag. The next day, the nitrocellulose membrane was taken out of the bag, and washed with a solution of 2 X SSC and 0.1% SDS for 15 minutes and with a solution of 0.1 X SSC and 0.1% SDS for 15 minutes, increasing the temperature stepwise, until the cpm value of the membrane reached about 1,000 cpm. After washing, the washing solution was removed by filter paper, and then the membrane was subjected to autoradiography. The plaque containing the desired gene was identified by exposure of a Fuji X-ray film. The genes were cloned by repetition of the above plaque hybridization.

20 X SSC contains 0.3 M sodium citrate (pH 7.0) and 3 M NaCl; 20 X SSPE contains 0.2 M sodium phosphate, 20 mM EDTA and 3 M NaCl (pH 7.4); and Denhardt's solution contains 1% Ficoll, 1% polyvinylpyrrolidone and 1% BSA (Penlex Fraction VI).

##### (3) Determination of Nucleotide Sequence (Sequencing)

All of the five isolated clones A4, A5, B9, C4 and M3 were each subcloned into plasmid pUC19. In subcloning each clone into plasmid pUC19, subcloning was carried out utilizing a restriction enzyme recognition site which produced a fragment hybridized with the probe for each clone. However, for cloning clone A4, a commercial BglII linker was used to ligate a *Sma*I site.

The plasmids were each transformed into competent cell HB101 (E. coli) prepared by the rubidium chloride method to obtain five kinds of transformants E. coli HB101/pXbr43 (coding for protein M3), E. coli HB101/pXbr44 (coding for protein A4), E. coli HB101/pXbr45 (coding for protein A5), E. coli HB101/pXbr46 (coding for protein B9) and E. coli HB101/pXbr47 (coding for protein C4), respectively.

For determination of the nucleotide sequence, a deletion mutant of each clone was prepared, and the shortest of fragment hybridized with the probe was selected. The nucleotide sequence was determined from pUC19 by the direct Sanger method (for the dideoxy method).

For translation of the nucleotide sequence to an amino acid sequence or for screening of homology, a software for genetic analysis (GENETYX, Nippon SDC) was used.

nucleotide	Homology at Nucleic Acid Level				
	Rat <i>Act βA</i> , %	Rat <i>Act βA</i> , %	Human TGF <i>β2</i> , %	xVgr %	M3 %
A5	70.3 (101)	47.5 (314)	43.8 (169)	48.5 (171)	54.7 (258)
A4	69.5 (0.5)	-	-	-	55.4 (251)
M3	63.6 (332)	53.9 (672)	33.1 (689)	-	-

In the above table, numerical values in parentheses indicate the length compared (bp).

nucleotide	Homology at Amino Acid Level				
	Rat <i>Act βA</i> , %	Rat <i>Act βA</i> , %	Human TGF <i>β2</i> , %	xVgr %	M3 %
A5	58.8 (34)	44.1 (34)	37.2 (43)	50.0 (38)	26.0 (77)
A4	41.3 (63)	44.1 (34)	39.5 (43)	52.6 (38)	30.3 (66)
M3	50.3 (149)	49.4 (162)	32.8 (128)	40.6 (106)	-

In the above table, numerical values in parentheses indicate the length compared (bp).

#### Example 2

##### Preparation of *Xenopus laevis* Unfertilized Egg-Derived DNA Library

##### (1) Preparation of *Xenopus laevis* BMP-2A Probe

A probe was prepared by fragmentation of chromosome DNA Xbr14 coding for *Xenopus laevis* BMP-2A with restriction enzymes *Pst*II and *Hind*III, and three kinds of cDNAs, Xbr22, Xbr23 and Xbr41 were isolated by screening of a *Xenopus laevis* unfertilized egg cDNA library by a hybridization method. The comparison with the structure of the *Xenopus laevis* BMP chromosome DNA already isolated revealed that Xbr22, Xbr23 and Xbr41 coded for proteins having homology with *Xenopus laevis* BMP-2A, *Xenopus laevis* BMP-2B and mouse Vgr-1 reported by Lyon et al. [Proc. Natl. Acad. Sci. U.S.A. 86, 4554-4558 (1989)], respectively.

The *Xenopus laevis* unfertilized egg cDNA library was provided by the Salk Institute (C. Kintner). This

library was prepared based on  $\lambda$ gt10. This recombinant phage was amplified by infection with *E. coli* NM514. Specifically, the phage was mixed with excess NM514 to allow NM514 to adsorb the phage at 37 °C for 10 minutes. Then, the mixture was plated on NZYM medium (containing 13% agar), followed by incubation overnight.

## (2) Screening

The total number of the phage clones was estimated to be about 1,200,000 from the number of the plaques produced in a dish. As a probe (DNA used for detection of a desired gene by hybridization), there was used a DNA fragment (185 bp) obtained by cleaving  $\lambda$ gt10 with restriction enzymes *Pst*I and *Hind*III and labeled with  $\gamma$ -32P by a random priming method. The plaques transcribed from the dish to a nitrocellulose membrane were returned to neutrality (0.2 M Tris, 0.6 M NaCl, pH 7.4) through alkali treatment (immersion in 0.1 N NaOH, 0.6 M NaCl for 30 seconds). After completion of the treatment described above, the membrane was heated in a vacuum thermostat at 80 °C for 1 hour. After heating, the membrane was immersed in a hybridization solution (50% formamide, 5 X Denhardt's solution, 5 X SSPE, 0.1% SDS, 100  $\mu$ g/ml denatured salmon sperm DNA) to incubate it at 42 °C for 4 hours. Then, the membrane was allowed to stand in the mixture solution of the above hybridization solution and the DNA probe at 60 °C overnight. This procedure was carried out in a plastic bag. The next day, the nitrocellulose membrane was taken out of the bag, and washed with a solution of 2 X SSC and 0.1% SDS for 15 minutes, increasing the temperature stepwise, until the cpm value of the membrane reached about 1,000 cpm. After washing, the washing solution was removed by filter paper, and then the membrane was subjected to autoradiography. The plaque containing the desired gene was identified by exposure of a Fuji X-ray film. The genes were cloned by repetition of the above plaque hybridization.

20 X SSC contains 0.3 M sodium citrate (pH 7.0) and 3 M NaCl; 20 X SSPE contains 0.2 M sodium phosphate, 20 mM EDTA and 3 M NaCl (pH 7.4); and Denhardt's solution contains 1% Ficoll, 1% polyvinylpyrrolidone and 1% BSA (Pentax Fraction V).

## (3) Determination of Nucleotide Sequence (Sequencing)

All of the three isolated clones Xbr22, Xbr23 and Xbr41 were each subcloned into plasmid pUC18, in subcloning each clone into plasmid pUC18, subcloning was carried out utilizing a restriction enzyme recognition site which produced a fragment hybridized with the probe for each clone.

The plasmids were each transformed into competent cell HB101 (*E. coli*) prepared by the rubidium chloride method to obtain three kinds of transformants *E. coli* HB101/pXbr22 (coding for *Xenopus laevis* BMP-2A), *E. coli* HB101/pXbr23 (coding for *Xenopus laevis* BMP-2B) and *E. coli* HB101/pXbr41 (coding for protein *Xenopus laevis* Vgr-1), respectively.

For determination of the nucleotide sequence, a deletion mutant of each clone was prepared, and the shortest fragment that hybridized with the probe was selected. The nucleotide sequence was determined from pUC19 by the direct Sanger method (or the dideoxy method).

For translation of the nucleotide sequence to an amino acid sequence or for screening of homology, a software for genetic analysis (GENETYX, Nippon SDC) was used.

Figs. 2(6) to 2(8) show the respective nucleotide sequences, and Figs. 4(VI) to 4(VIII) show the respective amino acid sequences.

## Example 3

In order to examine the biological activity of the *Xenopus laevis* BMP-related gene products, each of Xbr22, Xbr23 and Xbr41 cDNAs was inserted into expression vector pCDM8 (Invitrogen, U.S.A.) for animal cells, and expressed in a COS cell/African green monkey kidney cell. The resulting culture supernatant was used for determination of the biological activity.

Each of the Xbr22, Xbr23 and Xbr41 cDNAs to which XhoI linkers were ligated at both ends thereof was inserted into the XhoI restriction enzyme-cleaving site of pCDM8 to use it for transfection (introduction of DNA).  $3 \times 10^4$  cells were subcultured in a 100 mm diameter plastic dish, and the medium was removed after 24 hours, followed by washing once with 10 ml of TBS (Tris-buffered saline). 300  $\mu$ l of a DNA solution (1.5  $\mu$ g DNA) diluted with TBS was mixed with 300  $\mu$ l of a 0.1% DEAE-dextran solution, and the combined

solution was added dropwise to the cells. After standing at ordinary temperature for 15 minutes, the cells were washed once with 300  $\mu$ l of TBS, and then incubated in Dulbecco's modified Eagle's medium (DMEM, containing 10% FBS, 100 U/ml penicillin, 100 mcg/ml streptomycin and 100 U/ml chloroquine). After 3 hours, the cells were washed twice with TBS and incubated in DMEM (containing 10% FBS, 100 U/ml penicillin and 100 mcg/ml streptomycin). After 24 hours, the cells were washed three times with TBS and incubated in DMEM (containing 100 U/ml penicillin and 100 mcg/ml streptomycin) for 4 days, followed by recovery of the medium. The recovered medium was centrifuged at 2,000 rpm for 5 minutes to obtain a culture supernatant.

The culture supernatant thus obtained was used for determination of the biological activity as a sample containing *Xenopus laevis* BMP-2A, BMP-2B or protein Vgr-1. Namely, each of the samples was added to the medium of rabbit chondrocytes in monolayer cultures (Y. Kato et al., *Exp. Cell Res.* 130, 73-81 (1980); Y. Kato et al., *J. Biol. Chem.* 265, 5903-5909 (1990)) to examine their effect on the synthesis of proteoglycan, the main component of a cartilage matrix. As a result, the control in which the COS cell was transfected with the expression vector alone and the medium conditioned by untreated COS cells did not affect the synthesis of proteoglycan, as shown in the following table. In contrast, the above three kinds of proteins obtained in the present invention strongly promoted the synthesis of proteoglycan by the cartilage cells. The maximum activity of *Xenopus laevis* BMP-2A, BMP-2B and Vgr-1 was stronger than that of TGF- $\beta$ 1. The synthesis of proteoglycan was determined by measuring  $^{35}$ S-sulfate incorporation into glycosaminoglycans (Y. Kato et al., *Exp. Cell Res.* 130, 73-81 (1980); Y. Kato et al., *J. Biol. Chem.* 265, 5903-5909 (1990)). These results show that the BMPs of *Xenopus laevis* promote the differentiation of cartilages, and suggest that the BMPs of other animals have similar effects. The BMPs are therefore expected to be applied to therapeutic agents for healing acceleration of fractures and for various diseases of cartilages and bones (such as arthritis and osteoporosis).

\* Kind of Cell

\* Rabbit costal chondrocytes maintained on 6-mm diameter plastic wells.

\* Kind of Marker

\* S  $\mu$ Ci in 100  $\mu$ l medium per well

\* Kind of Medium

\* A 1:1 (V/V) mixture of DMEM and Ham's F-12 medium supplemented with 0.3% fetal bovine serum.

1

## 35

10

## 50

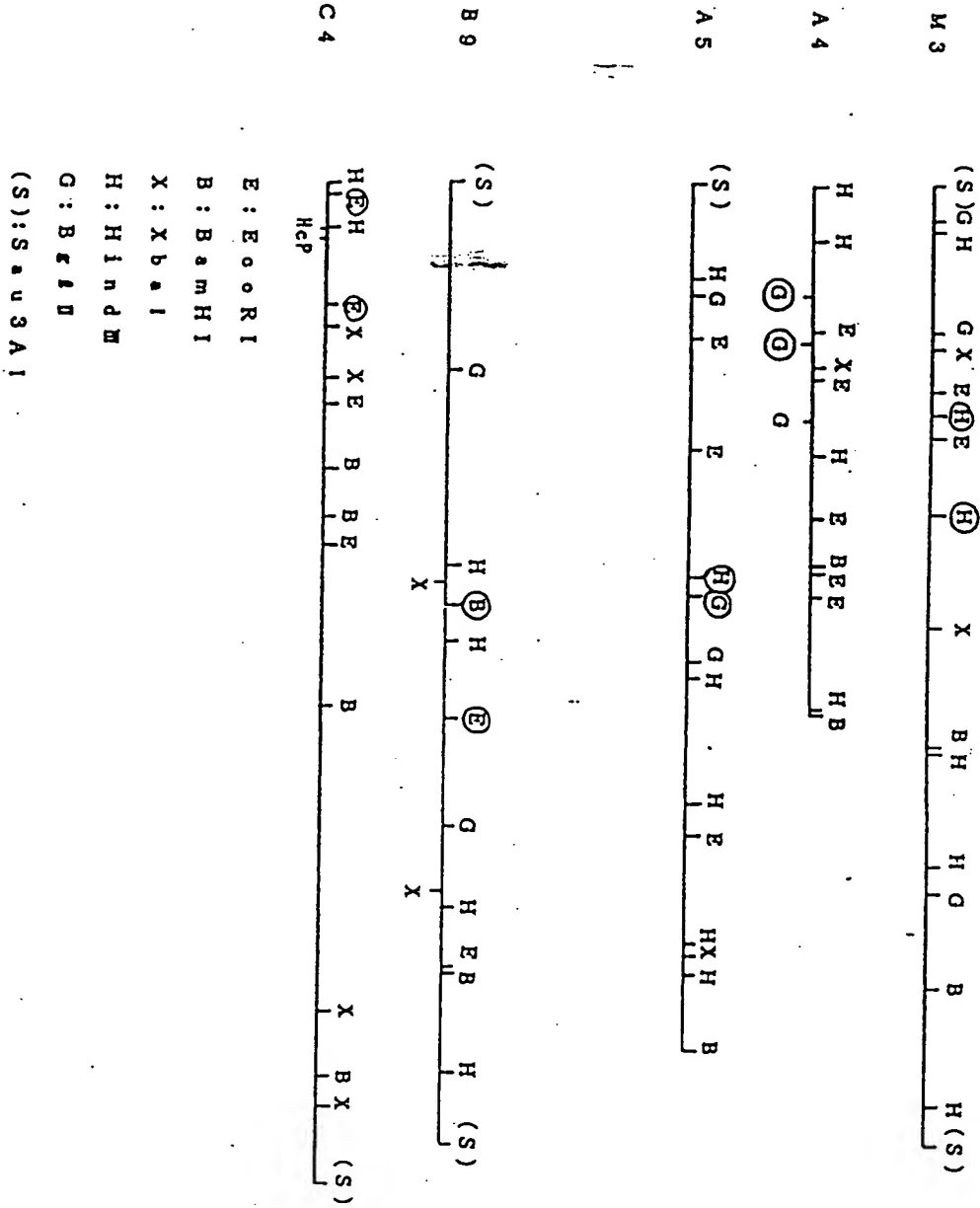
- 55

5

- 30

35

Fig. 1





F 1 8 . 2 ( 1 ) - 1  
B 9

TTCCAGTCTGATTGCAAGTGGATACCTTACATACATACATACGGAAGAAACAGAAATA  
AGTATANNTAAGTGATTAGCATAGATATCGGTATACNTGATCTNTGTCANTG  
TCAATCTAATGTAAAGCAAAACTCTTAAGNTCATGGCTAATACTGTNTGNTGCCCTT  
TCGTGAAATTCAGGATTCATCCAGAAATTAATCGGCTTGAATTCCTCAAGAGGCA  
GTAACTTTTAAATTTATGTAAGCGGACCTTTGGCTGTTCTTAAGTTCCTCAAGGCTTA  
ATCGAAGTAAATTCAGCAACCTGATTAATGATACCTTACCTTAAGAGGCTTAAGGCTTA  
AGCGTGGATTCAGAGCAACCTTAAGAGGCTTAATGATACCTTACCTTAAGAGGCTTA  
GGAAGAGTGGATTCAGATTAATTCAGATTAATTCAGATTAATTCAGATTAATTCAGATTA  
GCAATCAATTCAGATTAATTCAGATTAATTCAGATTAATTCAGATTAATTCAGATTA  
CTGTCTTGGCTTGGATTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGG  
GTAGAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTAAGTA  
CAGATGAAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTA  
TTGTGCTGAAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTAAGAGGCTTA

16

F 1 E . 2 ( 2 )

M 3

A G C T T A C G 1 0 T T G G A T T G 2 0 A C T G T G A T G G 3 0 A T G C C A A G A 4 0 T G T G C A G T 5 0 T T A G C C A A T 6 0  
 A G C T T A C G 1 0 T T G G A T T G 2 0 A C T G T G A T G G 3 0 A T G C C A A G A 4 0 T G T G C A G T 5 0 T T A G C C A A T 6 0  
 C A T A A T T C C C A C C A G G C A T T C C T G G T A G 1 0 A G A G C C A L Y A V A T G A T G A 1 0 A A G T G A C C A  
 T G T A C A L Y A G A G A G T C T T A A C C T G T G A T C G 1 0 A A C T C C C A A T T C T G T G C T G T A G G A A A G A C C A  
 T T A T G T A A C C T T C A A G G A T T 1 0 T T G G T G G A A T G A T T G G A T T 1 0 T A A A C C A G G 1 0 A G G A T T A T T  
 G A T A A A T T A T T C G C A T G G C C C T T G C C C A A T G C A T A T C A T A G G 1 0 A G C C C A G G 1 0 A G G A T T A C G A G  
 C T C A T C C A C C A C C A G G T A L L A A A T C T C A T T A A G G C C A A C C A A T A T C C A G A G A T T G A A  
 C T C A T G C C C T G T G T C T A C C C A A A G G C C G C C T T G T C C A T T G C C A T T G C C A T T G C C A T T G C C A  
 C A C G T T C C A A G A C T G A C A T T G C C G A T A T G A T T G T G G A A G C C T G T G G 1 0 G T A G C T A G G  
 C T T G G C C T A C A T C A G T T T G G A C A T T T A C A A A A A G A G G A G C T G G C T T T C T C T T  
 C A T T A T T T G C T C G A G A T G C T T A G A C A G G T G A A G A C A A G C T G A A A A A C T G A T T C C A C A T  
 T C A C T T A T C A A A T T C A T G G G A A T C G T A A C A N G A T C A N C C T C T C T A A A C G G A T C T A G G G A  
 G C T G T A G C A A T A N C T N T C C A G C A G G

F i s . 2 ( 3 ) - 1

C 4

AGAGCAGGTC<sup>10</sup>AGAGCCCT<sup>20</sup>TGTGANA<sup>30</sup>GTGACAGCAGCA<sup>40</sup>ATTGCATCGGATTANTATT<sup>50</sup>TAC<sup>60</sup>  
 GACATTGTCAGGSCAGCGGNNNCTGGSCTSCCGGGGGCCCTGTTGTGAGACTATTGAC<sup>120</sup>CA  
 ArgGlyProValValArgLeuLeuAspThr  
 CCAAACTGGTACATCATATA<sup>140</sup>GAAGAACAATGGGAAGAAGTTTGTATGTAGCGCGGCAATTG<sup>180</sup>  
 LysLeuValHisHisAsnGluSerLysTrpGluSerPheAspValAlaProAlaIleAla  
 CGCGGTGGATTGACACATAA<sup>200</sup>ACAGCCCTAACCATGGGTGTGTGTGAAGTTACTCAGCTTGG<sup>240</sup>  
 ArgTrpIleAlaHisLysGlnProAsnHisGlyPheValValGluValThrHisLeuAsp  
 ACAATGACAA<sup>250</sup>AAATGTGCTTAAAGAAGCATGTGAGGATTAGTAGGTCTTTAACCCGGATA<sup>300</sup>  
 AsnAspLysAsnValProLysLysHisValArgIleSerArgSerLeuThrProAspLys  
 AAGATAACTGGCCTCAGATTA<sup>320</sup>CGGCCATTGTGTAACTTTTAGCCATGATGGTAAAGGAC<sup>360</sup>  
 AspAsnTrpProGlnIleArgProLeuLeuValThrPheSerHisAspGlyLysGlyHis  
 ATGCTCTTCACAA<sup>380</sup>AGACAA<sup>390</sup>AGCGCCAAAGCTAGGCACAA<sup>400</sup>ACACGTAA<sup>410</sup>ACGCCCTTAAAT<sup>420</sup>  
 AlaLeuHisLysArgGlnLysArgGlnAlaArgHisLysGlnArgLysArgLeuLysSer  
 CGAGCTGCAGAGGAGCATCCGTTGTACGTAGATTTCAGCGACGACGTTGGTTGGAATGACTGGA<sup>480</sup>  
 SerCysArgArgHisProLeuTrpValAspPheSerAspValGlyTrpAsnAspThrPhe  
 TTGTGCCCCACCTGGGTATCATGCGCTTTTAACTGCCACGGGGAATGTCCCTTTCCACTGG<sup>540</sup>  
 ValAlaProProGlyTrpHisAlaPheTrpCysHisGlyGluCysProPheProLeuAla

F 1 8 . 2 ( 3 ) - 2

550 560 570 580 590 600  
CAGACCATTTAAACTCTACCAACCATGCAATCGTACCAACTTTGGTGAACCTCTGTCAACA  
AspHisLeuAsnSerThrAsnHisAlaIleValGlnThrLeuValAsnSerValAsnThr  
610 620 630 640 650 660  
CAAAACATCCCCCAAAGCTTGCGTGCCCAAGCAACTGCGCATATCCCATGCTCTATC  
AsnIleProLysAlaLysCysValProThrGluLeuSerAlaIleSerMetLeuThrLeu  
670 680 690 700 710 720  
TTGATGAGAAATGAAAAAGTAGTATTAAAAAATTATCAAGACATGGTCCGTGGAGGGGTGCG  
AspGluAsnGluLysValIleValLeuLysAsnTyrGlnAspMetValValGluGlyCysGly  
730 740 750 760 770 780  
GATGCCGTTAGGCAGTTACGCGCAAGCCAGAGACACAAGAAAGATGACACCTTTAATATTCC  
CysArg\*\*\*  
790 800 810 820 830 840  
TTTGGAGACTATATTATGCTTTGAAAAAATGATGAACAACANTTATTTTGAAAAATATATT  
850 860 870 880 890 900  
ATGTCTACACGGAGGTTGGGAAGCAATATTTTAATCAGAGAAATATTCCTTTTATTAGT  
910 920 930 940 950  
TGTACATTTTATTAAGGGTTTGTACCCAGCACATGAAGTATTAATGATCAGATTGA

F i e . 2 ( 4 )

A 4

```

10      20      30      40      50      60
CCTGAGANTTAAGAAGTGTGGGATTTAACAGAACAGGACCGACCAATGAGAAAGCTA
70      80      90      100     110     120
TTTTCCTGTCTNTGGTAGGACCAAGAACGGGACNTGTTCTTCAATGAGATTAAAGCCA
PhePheAsnGluIleLysAlaArg

130     140     150     160     170     180
GGTCTGCCCAAGATGACAAAGACTGTCTATGAAATATTATTCATCAGAGGAGAAAGAGAC
SerGlyGlnAspAspLysThrValTyrGluPheAsnGlnArgLysArgLysArg
190     200     210     220     230     240
GAGCTCCTCTGTCAACTAGGCAAGGGAAGAGGCCCTAATAAGAAATTCAAAAGCAAGATGTA
AlaProLeuSerThrArgGlnGlyLysArgProAsnLysAsnSerLysAlaArgCysSer
250     260     270     280     290     300
GCAAGAAACCACTTCATGGTCAATTTCAGAGGATATGGGTTGGGATGATTGGATTATTGCCCC
LysLysProLeuHisValAsnPheLysAspMetGlyTrpAspAspTrpIleIleAlaPro
310     320     330     340     350
CTTGGAGTATGAGGCATATCATTTGTGAAGGGCTTTGTGAGTTCCCTCTGAGATCT
LeuGluTyrGluAlaTyrHisCysGluGlyLeuCySGluPheProLeuArgSer

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F i g . 2 ( 5 )

A 5

```

10      20      30      40      50      60
AAGCTTACTGGTGGTGTTCTTCCCATTCACAAGAGGAGGAAACTTGTTTAAGGAGATCA

70      80      90      100     110     120
GGGACACAGATTAAAGTCAATTGGAAATCCTAAATTCTTGGAGCCACCGGATTTCAGTCAACA
      1leLeuGlyAlaThrGlyPheSerGlnGln

130     140     150     160     170     180
GTCCATCGCCCAAGAGGAGATGGAAACGAACCAACTCTCCCCCACTAGGACAAATAATGGCA
      SerIleAlaLysArgArgTrpLysArgThrLeuProThrArgThrAsnAsnGlyLys

190     200     210     220     230     240
AGGTCATGGCGAAGAAATCCCAAACAAGGTGTAGCAAGAGCCCTTCTTGTCACACTTCAA
      GlyHisAlaLysSerLysThrArgCysSerLysProLeuLeuValAsnPheLys

250     260     270     280     290     300
GGAGTTGGGTTGGGATGACTGGATTATTGCTCCCTTGGAATTATGAAGCCTATCACTGCGA
      GluLeuGlyTrpAspAspTrpIleIleAlaProLeuAspTyrGluValaTyrHisCysGlu

310     320
GGGGTCTGTGATTTCGCCACTGAGATCT
      GlyValCysAspPheProLeuArgSer

```

Fig. 2 (6)  
p x b r 2 2 (BMP 2 A)

10 20 30 40 50 60 70 80 90 100  
GAATTCTCTCCCTCTCACC GGCTCTCGTCTCTACTCAGCTCCCGGGGACCCCGGCTGGACTGAGACACTCGCTGCCACTATGTGCGACAACCTACCGA

110 120 130 140 150 160 170 180 190 200  
CTGGGCTCGACTGGAGCGCGGACTTGTCTCCCTCTCTGGGGACCAAGGACTTGAACATAAGACTCGAGTGATTGTGGAAAAACACCGGGGAGCAGA

210 220 230 240 250 260 270 280 290 300  
AAACCCACATCGAGACACAACCTCGGGGACTAAATCGCTCAGGTTGACAATGGTCGCTGGGATCCACTCTCTGCTCCTGCTGCAAGTTTACCAGATCTTG  
M V A G I H S L L L L Q F Y Q I L

310 320 330 340 350 360 370 380 390 400  
CTGAGCGGCTGCACCGGGCTCGTCCAGAGGAAGGCAACGCAAGTATTCGGAATCCACTCGCTCGTCTCCGAGCAGTCCCAACAAGTCCCTCGACCAAT  
L S G C T G L V P E E G K R K Y S E S T R S S P Q Q S Q Q V L D Q F

410 420 430 440 450 460 470 480 490 500  
TTGAGCTTCGGCTGCTCAATATGTTGGCTTGAAGAGGAGGCGGACGCTGGCAAAAAATGTTGTGATCCCCCTACATGTTGAGCTTGTACCACTGCA  
E L R L L N M F G L K R R P T P G K N V V I P P Y M L D L Y H L H

510 520 530 540 550 560 570 580 590 600  
CTCGGCTCAGTTGGCGATGATCAAGGAAGTTCTGAGGTGGACTATCAGATGGAGCGGCTAGCAGAGCCAACAGTGAAGGAGCTTTACCATGAA  
S A Q L A D D Q G S S E V D Y H M E R A A S R A N T V R S F H H E

610 620 630 640 650 660 670 680 690 700  
GAATCCATGGAAGAAATTCAGAGTCTGGTGAGAAAACAATCCAACGATTTCTTCAACCTTTCTTCAATTCCAGATGAGGAGCTGGTCAAGCTTCTG  
E S M E E I P E S G E K T I Q R F F F N L S S I P D E E L V T S S E

710 720 730 740 750 760 770 780 790 800  
AGCTCCGGATTTTTCGAGAGCAGGTCCAAGAGCCATTAAGACTGACGGCAGCAAACTTCATCGGATTAATATTTATGACATTGTCAAGCCAGCGGCGGC  
L R I F R E Q V Q E P F K T D G S K L H R I N I Y D I V K P A A A

810 820 830 840 850 860 870 880 890 900  
TGCCTCCCGGGCCCTGTGTGAAGACTATTGGACACCAGACTGATCCATCATAATGAAAGCAATGGGAAAGTTTGTGATGACCGCGGCAATTACACGG  
A S R G P V V R L L D T R L I H H N E S K V E S F D V T P A I T R

910 920 930 940 950 960 970 980 990 1000  
TGGATTGCACATAACAGCCTAACCATGGGTTTGTGTGTAAGTGAAGTCACTTGGACAATGACACAAATGTGCCAAGAGGCATGTGAGGATTAGTAGGT  
V I A H K Q P N H G F V E V T H L D N D I N V P K R H V R I S R S

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100  
CTTTAACCTGGATAAAGGCTACTGGCTCGGATACGGCCATTATTTGGTAACTTTTAGCCATGATGGCAAAGGACATGCTCTTCAAAAAGACAAAACG  
L T L D K G H V P R I R P L L V T F S H D G K G H A L H K R Q K R

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200  
GCAAGCTAGGCACAAACAGTAAACGCCCTTAAATCGAGCTGCAGGAGGCATCCGTTGTACGTAGATTTCAGTGACGTTGGTTGGAATGACTGGAATTGT  
Q A (R) H K Q R K R L K S S C (R) H P L Y V D F S D V G V N D V I V

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300  
GCCCCACCTGGGTATCATGCTTTTACTGCCACGGGAATGTCTTTTCCACTGGCAGACCAATTAACCTACAAACCATGCAATCGTACAAACCTTTGG  
A P P G Y H A F Y C H G E C P F P L A D H L N S I N H A I V Q T L V

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400  
TGAATTCGTCAACACAAACATTCCCAAGCTTGTGCTGCTCCACAGAACTCAGTGCCATCTCCATGCTCTATCTTGATGAGAATGAAAAAGTAGTATT  
N S V N T N I P K A C C V P T E L S A I S M L Y L D E N E K V V L

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500  
AAAGAATTATCAAGACATGGTCTGGAGGGGTGCGGGTCCGTTAGGCGGGGACACAAAGCCAGAGACAAGAAAGCTGACACTTTAATATTTCCTTTTG  
K N Y Q D M V V E G C G C R \*

1510 1520 1530 1540 1550 1560 1570 1580 1590 1600  
GAGACTATATTTATGCTTTGAAAAATGATGAAACAATTATTTTAAAAATATATTTATGCTACACGGAGGCTGGGAAGCAATATTTTAAATCAGAGAAAT

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700  
ATTCTTTTTAGTTGTACATTTTTATAAGGTTTGTACCCAGCAGATGAAGTATAATGGTCAGATTCTATTTTGTATTTATTTACCATTTATAACCACTT

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800  
TTTAAGGAAAAAATAGCTGTTTGTATTTATATGTAATCAACAGAGAAAAATAGGGTTTGTAAATATGTTACTGAAAGTGTTTTTCTTCTTTTTT

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900  
TAAATTATGTTATACAGCTGGTTATATGGCAAGTTTTTATATTTCTATAAAGCTAATTTCAAGGTCAATTAGTTATAAACTTGATGATGTTGGTTC

1910 1920 1930 1940 1950 1960 1970 1980 1990  
ATTGGTAAATCCCTCATATTGTGCAATTAACATGCATTTTTATAATGTACGAAGTCCAGTCCATTGTGCAATTGCTTTGCAAAATTAGAAATC



F i g . 2 (7)  
p x b r 23 (BMP 2B)

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10      20      30      40      50      60      70      80      90      100
GGAATTCCGGCCCCACTGAGCTTTTCCACACATTTTTGTGTCCAACATTGGCTGTCAAGAATCATGGAATGTTTTCTATGCCCTTGTTTTCTGTCAAGA

110     120     130     140     150     160     170     180     190     200
CATCATGATTCCTGGTAACCGAATGCTGATGGTCATTTTATTAAGCCAAGTCTGCTCGGAGGCACTAACTATGCCAGCCTGATACCTGACACGGGCAAG
M I P G N R M L M V I L L S Q V L L G G T N Y A S L I P D T G K

210     220     230     240     250     260     270     280     290     300
AAGAAAGTCGGGCGGACATTCAGGGAGGAGTCCGAGGTCGCTCAGAGCAATGAGCTCTTGGGGATTTGAGGTGACGCTGCTGCAGATGTTCCGGAC
K K V A A D I Q G G G R R S P Q S N E L L R D F E V T L L Q N F G L

310     320     330     340     350     360     370     380     390     400
TCCGCAAGCGGCGCAGCCAGTAAGGATGTGGTGGTTCGCGCTTATATGCGCGACCTGTACAGGCTTCAGTCAGCGGAGGAGGAGGATGAACTGCACGA
R K R P Q P S K D V V V P A Y M R D L Y R L Q S A E E E D E L H D

410     420     430     440     450     460     470     480     490     500
TATCAGCATGGAGTACCCGAGACACCAGCCGCGCAACACCGTGAGGAGCTTCCATCAGGAGGAACATTTGGAGATCTACCAGGCACAGAAGAA
I S M E Y P E T P T S R A N T V R S F H H E E H L E N L P G T E E

510     520     530     540     550     560     570     580     590     600
AATGGAATTTCCGTTTTGTGTCAACCTCAGCAGCATTCCAGAGAATGAGGTGATTTCTTCAGCAGAACTGAGACTCTATAGAGAACAAATAGACCATG
N G N F R F V F N L S S I P E N E V I S S A E L R L Y R E Q I D H G

610     620     630     640     650     660     670     680     690     700
GTCACCGTGGGATGAGGGTTTCCACCGGATAAATATATGAAGTTATGAAACCCATCAGCAACCGGACACATGATAAATAGGCTGCTGGACACGAG
P A V D E G F H R I N I Y E V M K P I T A N G H M I N R L L D T R

710     720     730     740     750     760     770     780     790     800
GGTAATCCACCACAATGTGACACAGTGGGAAAGTTTGTATGAAGCCTGCAATTATGAGGTGGACCTGGATAAACAGATAAACCATGGGCTTGCCATT
V I H H N V T Q V E S F D V S P A I M R V T L D K Q I N H G L A I

810     820     830     840     850     860     870     880     890     900
GAGGTCAITTCACCTCAACCAACAAAACTTATCAGGGGAAGCATGTAAGGATAAGTCGATCTTTATTACCTCAAAAGGATGCAGACTGGTCACAGATGA
E V I H L N Q T K T Y Q G K H V R I S R S L L P Q K D A D V S Q M R

910     920     930     940     950     960     970     980     990     1000
GACCACITTTAATTACATTCAGCCATGATGGCAGGGGCGATGCAGTACTAGGAGGTCAAAAAGAAGTCCAAAACAGCAGAGACCCGTAAAAAAATAA
P L L I T F S H D G R G H A L T R R S K R S P K Q Q R P R K K N K

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
ACACTGCCGGAGACATTCCTTTATGTGGATTTCAGCGATGTGGGCTGGAATGATTGGATTGTGCACCTCCTGGATACCAGGCCITTTACTGCCATGGA
H C R R H S L Y V D F S D V G V N D V I V A P P G Y Q A F Y C H G

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
GATTGTCCATTTCCCTTGGCTGATCACCTAACTCACTAACCATGCTATTGTACAACTCTGGTAACTCTGTAACTCAAGCATCCCAAAAGCATGCT
D C P F P L A D H L N S T N H A I V Q T L V N S V N S S I P K A C C

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
GCGTCCCCACAGAAGTGAAGTCTATCTCCATGCTTTATTTGGATGAATATGACAAAGTCGTCCTTAAAAACTACCAGGAGATGGTGGTGGAAAGGGTGTGG
V P T E L S A I S M L Y L D E Y D K V V L K N Y Q E X V V E G C G

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
GTGCCGTTGAGTCTGAGATCCAAACAAAAGACTGTTAACGGCTGGACTTCTTTCCACTGAACATTCACCTTGACCTTTATATGACTTTTATGTGTAAT
C R *

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
GTTTTTTTGACAATATGATCATATATTTTGACAAAATATATTTATAACTACGTATTAAGAAAAAATAAATAAAGTCATTATTTTAAACATAA

1510    1520    1530    1540    1550
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACCGGAATTC

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F i g . 2 (8)  
p x b r 4 1 (V e r 1)

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10      20      30      40      50      60      70      80      90      100
GAATTCGATATGGAATGTAATAAATACTGGTGAATTATGGGAAGTCCGACACAGACCCTAACTTCAGCATCTTATCTTTTGACAAAATGAATGCTTTGAC
M N A L T

110     120     130     140     150     160     170     180     190     200
AGTAAAGAGAAGATTGCCGTGCTGCTTTTCTTTTTCACATTTCACTGAGTTCCATCTCGTCAAATACAATATTGGAGAATGATTTCCTCTAGTTT
V K R R L P V L L F L F H I S L S S I S S N T I L E N D F H S S F

210     220     230     240     250     260     270     280     290     300
GTCCAGAGAAGACTAAAGGCCACGAACGACAGAGAGATTCAAAAAGAGATCTTGACTATTTAGGTTTGCAACACAGACCAAGGCCATATTTACGGGAGA
V Q R R L K G H E R R E I Q K E I L T I L G L Q H R P R P Y L P E K

310     320     330     340     350     360     370     380     390     400
AAAAGAAGCTGCACCATTTATTCATGATGGATTATACAATGCAGTAAATATTGAAGAGATGCATGCTGAAGATGTTTCTACAGCAATAAGCCGATCTC
K K S A P L F M M D L Y N A V N I E E M H A E D V S Y S N K P I S

410     420     430     440     450     460     470     480     490     500
CCTAAATGAAGCTTTTCACTGGCCACTGACCAAGAGAATGGCTTTCTTGCACATGCCGACACAGTTATGAGTTTGTCTAATTTAGTTGACAATGACAAC
L N E A F S L A T D Q E N G F L A H A D T V M S F A N L V D N D N

510     520     530     540     550     560     570     580     590     600
GAATTCGATAAAACTCTATCGCCAAAATTCAGTTTGATCTAACTGATATCCCACTTGGAGATGAAGTACAGCCGCTGAATTCGAATTTATAAAG
E L H K N S Y R Q K F K F D L T D I P L G D E L T A A E F R I Y K D

610     620     630     640     650     660     670     680     690     700
ATTATGTACAAAATAACGAGACATACCGGTCACCATCTACCGGTCGTTAAGAAGCAAGCCGACAAAGATCCTTATCTTTCCAGGTAGACTCAAGAAC
Y V Q N N E T Y Q V T I Y Q V L K K Q A D K D P Y L F Q V D S R T

710     720     730     740     750     760     770     780     790     800
CATCTGGGGCAGACAAAAGGGATGGCTGACGTTTGATATTACTGCAACTGGTAATCACTGGGTGATGAACCCACATTACAACCTTGGATTGCAGTTATCA
I V G T E K G V L T F D I T A T G N H V V M N P H Y N L G L Q L S

810     820     830     840     850     860     870     880     890     900
GTAGAGAGTATGGATATGCAAAATGTTAATCCAGGCTTGTGGGCTTGTGGAAAGAATGGTCCTCAAGACAAACAGCCATTTATGGTGGCATTCTTTA
V E S M D M Q N V N P R L V G L V G K N G P Q D K Q P F M V A F F K

910     920     930     940     950     960     970     980     990     1000
AGACCTCAGATATCCATCTCCGAGTGTTCGATCTACTAGCAATAAGCACTGGAATCAGGAAAGAGCCAAGACCTACAAGGAGCAAGATAATTTACCTCC
T S D I H L R S V R S T S N K H V N Q E R A K T Y K E Q D N L P P

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AGCAAAATATTACTGATGGCATCATGCCCTGGAAAACGTCGTTTTTAAAGCAAGCTTGCAAGAAACATGAAGTGTGTAAGTTTCCGCGATCTTGGT
A N I T D G I M P P G K R R F L K Q A C K K H E L F V S F R D L G

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
TGGCAAGACTGGATAATTGCACCTGAAGGATATGCTGCCCTACTATTGTGATGGAGAATGTGCTTTCCCACTTAACCTTTTCATGAATGCCACAAACCATG
V Q D W I I A P E G Y A A Y Y C D G E C A F P L N S F M N A T N H A

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CCATTGTACAAACGTTGGTACATTTTATTAACCCAGAGACTGTCCCTAAGCCATGCTGTGCACCAACTCAGCTCAATGGTATTTCTGTTTTTACTTTGA
I V Q T L V H F I N P E T V P K P C C A P T Q L N G I S V L Y F D

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TGACAGTGCCAAATGTTATATTAAGAAATACAAAATATGGTGGTTCAAGCCTGTGGTTGCCATTGACAATAGCAGTTATTCGTGTTTTAACAGTCATTT
D S A N V I L K K Y K N M V V Q A C G C H *

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TAATGGTATTGTCCTTATCGTTTATTTTAAAGTAGAGATACTTGACCATCACACTTAAAAAATGCATTGTACACCTTAACGGATGAAAAGATTTTGTIT
TTGCAATATTCGGAATTC

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## F i e . 3

#A ARQSEDPHRRRR--TGLECDGKVNICKKQFVVSFKDIGWNDWI IAPSGYHANYCEGECPSHIA  
 B9(1) . . . TDE. . . . . KK. . . . . S. . . . . H. Y. . . . . S. . . . . P. . . . . D. . . . .  
 M3(U) AK-VHEQS. HATK. --S. N. . QNS. L. . R. DYY. D. . . . . K. E. . QI. . M. L. . M. . . .  
 #8 . . LGDSR-. . I. K. --. . . . . RTS. L. . RQ. . . ID. RL. . . . . T. YG. . . . . S. . . . .  
 BMP2A K. EKQAK. KQ. K. --. . . . . SS-. . KRHPY. D. S. V. . . . . V. . P. . . . . F. . H. . . . . PPL. D  
 C4(W) K. . KRQAR. KQ. K. --. . . . . SS-. . RRHPY. D. S. V. . . . . V. . P. . . . . F. . H. . . . . PPL. D  
 DPFC . . . -HARRPT. . KN---H---DDT-. . RRHSY. D. S. V. . . . . DD. . V. . L. . D. Y. . H. K. . FPL. D  
 Vg1 TLNPLRCKRP. . K. SYSKLPFTAS. . K. RHY. E. . V. . QN. V. . Q. . M. . . . . Y. . . . . YPLTE  
 A4(W) R. KRAPLST. --QGKRPKNKS. AR-. . S. . PLH. N. . . M. . D. . . . . LE. E. YH. . . L. EFPLRS  
 A5(V) R. WKRTTLP. TNGK. HAKKS. TR-. . S. . PLL. N. . EL. . D. . . . . LD. F. YH. . . V. DFPLRS  
 CONSENSE . . . . . C. . . . . F. . . . . GW. W. . . P. . . . . C. G. C. . . . .

#A TSGSSLSFHSTVINHYRMGHSFANLKS CCVPTKLRPM SMLYDDGQNI IKKDIQNMIVEECGS  
 B9(1) . T. . . . . Q. . L. . Q. . . . . TSI. . . . . S. . . . . A. . . . . . . . . . .  
 M3(U) AP. TAA. . . T. . L. LIK--ANNIQTAVN. . . . . R. . L. . . . F. RNN. VL. T. . AD. . . . A. . . .  
 #8 VP. AS. . . TA. V. Q. . . . . LN. -GPVN. . . I. . . . . SS. . . . . F. . EY. . V. R. VP. . . . . A  
 BMP2A H--LNSTN. AI. QTLVN--SVNSK-IP. A. . . . . E. SAI. . . . L. ENKVV. L. NY. D. V. . G. . . R  
 C4(W) H--LNSTN. AI. QTLVN--SVNTN-IP. A. . . . . E. SAI. . . . L. ENKVV. L. NY. D. V. . G. . . R  
 DPFC H--FNSTN. AV. QTLVN--NMNPGK-VP. A. . . . . QLDVA. . . . LN. QSTV. L. NY. E. T. VG. . . R  
 Vg1 I--LNG. N. AILQTLVH--SIE. EDIPLP. . . . . MS. I. . . . F. . . . . NND. VVLRHYE. . A. D. . . . R  
 \*\*\*

CONSENSE . . . . . H. . . . . . . . . . . CC. P. . . . . ML. . D. . . . . . . . . . . M. V. . CGC.

F i g . 4 - 1

(VI) BMP2A

M V A G I H S L L L L Q F Y Q I L  
L S G C T G L V P E E G K R K Y S E S T R S S P Q Q S Q Q V L D Q F  
E L R L L N M F G L K R R P T P G K N V V I P P Y M L D L Y H L H  
S A Q L A D D Q G S S E V D Y H M E R A A S R A N T V R S F H H E  
E S M E E I P E S G E K T I T U R F F F N L S S I P D E E L V T S S E  
L R I F R E Q V Q E P F K T D G S K L H R I N I Y D I V K P A A A  
A S R G P V V R L L D T R L I H H N E S K V E S F D V T P A I T R  
V I A H K Q P N H G F V V E V T H L D N D T N V P K R H V R I S R S  
L I L D K G H V P R I R P L L V T F S H D G K G H A L H K R Q X R  
Q A R H K Q R K R L K S S C R R H P L Y V D F S D V G V N D V I V  
A P P G Y H A F Y C H G E C P F P L A D H L N S T N H A I V Q T L V  
N S V N T N I P K A C C V P T E L S A I S M L Y L D E N E K V V L  
K N Y Q D K V V E G C G C R \*

F i g . 4 - 2

(VI) BMP2B

M I P G N R M L N V I L L S Q V L L G G T N Y A S L I P D T G K  
K K V A A D I Q G G G R R S P Q S N E L L R D F E V T L L Q M F G L  
R K R P O P S K D V V V P A Y M R D L Y R L Q S A E E E E D E L H D  
I S M E Y P E T P T S R A N T V R S F H H E E H L E N L P G T E E  
N G N F R F V F N L S S I P E N E V I S S A E L R L Y R E Q I D H G  
P A V D E G F H R I N I Y E V M K P I T A N G H M I N R L L D T R  
V I H H N V T Q V E S F D V S P A I M R V T L D K Q I N H G L A I  
E V I H L N Q I K T Y Q G K H V R I S R S L L P Q K D A D V S Q M R  
P L L I T F S H D G R G H A L T R R S K R S P K Q Q R P R K K N K  
H C R R H S L Y V D F S D V G V N D V I V A P P G Y Q A F Y C H G  
D C P F P L A D H L N S T N H A I V Q T L V N S V N S S I P K A C C  
V P T E L S A I S M L Y L D E Y D K V V L K N Y Q E M V V E G C G  
C R =

F i g . 4 - 3

(M) (Vgr1)

M N A L T

V K R R L P V L L F L F H I S L S S I S S N T I L E N D F H S S F  
V Q R R L K G H E R R E I Q K E I L T I L G L Q H R P R P Y L P E X  
K K S A P L F M H D L Y N A V N I E E M H A E D V S Y S N K P I S  
L N E A F S L A T D Q E N G F L A H A D T V M S F A N L V D N D N  
E L H K N S Y R Q K F K F D L T D I P L G D E L T A A E F R I Y K D  
Y V Q N N E T Y Q V T I Y Q V L K K Q A D K D P Y L F Q V D S R T  
I V G T E K G V L T F D I T A T G N H V V M N P H Y N L G L Q L S  
V E S M D K Q N V N P R L V G L V G K N G P Q D K Q P F M V A F F K  
T S D I H L R S V R S T S N K H V N Q E R A K T Y K E Q D N L P P  
A N I T D G I M P P G K R R F L K Q A C K K H E L F V S F R D L G  
V Q D V I I A P E G Y A A Y Y C D G E C A F P L N S F M N A T N H A  
I V Q T L V H F I N P E T V P K P C C A P T Q L N G I S V L Y F D  
D S A N V I L K K Y K N M V V Q A C G C H \*

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